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Anthocyanin occurrence in the root peels, petioles and flowers of red radish (*Raphanus sativus* L.)

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Abstract

Three novel acylated pelargonidin 3-sophoroside-5-glucosides were isolated from the root peels, petioles and flowers of red radish, *Raphanus sativus* 'Cherry Mate', in addition to five known anthocyanins namely, pelargonidin 3-sophoroside-5-glucoside, pelargonidin 3-[2-(glucosyl)-6-(*trans*-*p*-coumaroyl)-glucoside]-5-glucoside, pelargonidin 3-[2-(glucosyl)-6-(*trans*-feruloyl)-glucoside]-5-glucoside, pelargonidin 3-[2-(glucosyl)-6-(*trans*-*p*-coumaroyl)-glucoside]-5-(6-malonylglucoside) and pelargonidin 3-[2-(glucosyl)-6-(*trans*-feruloyl)-glucoside]-5-(6-malonylglucoside). The structures of three new acylated anthocyanins were shown to be pelargonidin 3-*O*-[2-*O*-(β-D-glucopyranosyl)-6-*O*-(*trans*-caffeoyl)-β-D-glucopyranoside]-5-*O*-(6-*O*-malonyl-β-D-glucopyranoside), its demalonyl derivative, and pelargonidin 3-*O*-[2-*O*-(β-D-glucopyranosyl)-6-*O*-(*cis*-*p*-coumaroyl)-β-D-glucopyranoside]-5-*O*-(6-*O*-malonyl-β-D-glucopyranoside). These pigments were the main components present not only in the root but also in the petioles and flowers of red radish. *p*-Coumaroyl anthocyanins were the main pigments found in the root, petioles and flowers. Although the *trans*-*p*-coumaroyl form was abundant in all three plant organs, its *cis* form was present in very low amount within the root but in large amount in the flowers and petioles.

Keywords: *Raphanus sativus* L.; Cruciferae; Pelargonidin 3-sophoroside-5-glucoside; *trans*- and *cis*-*p*-coumaric acids; Caffeic; Ferulic and malonic acids

1. Introduction

Radish (*Raphanus sativus* L.) root anthocyanins have been characterized by several researchers [1–4], who determined the presence of pelargonidin 3-sophoroside-5-glucoside derivatives acylated with *p*-coumaric, caffeic and ferulic acids. Recently, 16 acylated anthocyanins from radish roots have also been fully characterized as 3-mono- or di-hydroxycinnamoyl (*p*-coumaric, caffeic and/or ferulic acid)-sophoroside-5-glucoside, 3-mono- or di-hydroxycinnamoyl (*p*-coumaroyl, feruloyl, or di-feruloyl)-sophoroside-5-malonylglucoside, 3-mono- or di-feruloyl-sophoroside-5-malonylglucosylglucoside and 3-di-feruloyl-sophoroside-5-glucosylglucoside of pelargonidin and 3-caffeoyl-feruloyl-sophoroside-5-glucoside of cyanidin [5–7] by spectroscopic analyses including mass spectrometry and NMR technique. However, the complete structural determination of an anthocyanin acylated with caffeic acid or *cis*-*p*-coumaric acid together with malonic acid from the radish has not been reported to date. Although the presence of anthocyanins in the root peels of red radish has been investigated, the occurrence of anthocyanins in the flowers and petioles has not been reported yet. In this paper, we wish to report the complete structural determination of three new acylated anthocyanins. During our investigation of the new anthocyanins, we recognized that the pigments previously discovered in the root were also present in the petioles and flowers.

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2. Experimental

2.1. Plant materials

Red radish (*R. sativus* L.) cultivars 'Cherry Mate' (Tohoku Co., Ltd., Utsunomiya, Japan) and 'Flamboyant Sabina' (Thompson & Morgan Ltd., England) were cultivated in a green house on the Experimental Farm of Minami-kyushu University from November 2006 to April 2007. The roots of 'Cherry Mate' and 'Flamboyant Sabina' were harvested in December 2006. The roots' peels were dried overnight at 40 °C and kept in a refrigerator at 4 °C. The petioles and flowers (acyanic flowers were excluded) of 'Cherry Mate' were harvested in January 2007 and April 2007, respectively. They were dried and kept along with the root peels until their use in the experiment.

2.2. General procedures

TLC was carried out on plastic coated cellulose sheets (Merck) using nine mobile phases: BAW (*n*-BuOH–HOAc–H_2O, 4:1:2, v/v/v), BuHCl (*n*-BuOH–2 N HCl, 1:1, v/v, upper layer), AHW (HOAc–HCl–H_2O, 15:3:82, v/v/v), 1% HCl and Forestal (HOAc–HCl–H_2O, 30:3:10, v/v/v) for anthocyanins, and BAW, APW (EtOAc–pyridine–H_2O, 15:7:5, v/v/v), EAA (EtOAc–HCOOH–H_2O, 5:2:1, v/v/v), BEW (*n*-BuOH–EtOH–H_2O, 4:1:2.2, v/v/v) and 15% HOAc (HOAc–H_2O, 15:85, v/v) for sugars and organic acid with UV light and aniline hydrogen phthalate spray reagent [8].

Analytical HPLC was performed on an LC 10A system (Shimadzu), using a Waters C18 (4.6 ∅ × 250 mm) column at 40 °C with a flow rate of 1 mL min^{-1} and monitoring at 530 nm. The eluant was applied as a linear gradient elution for 40 min from 20 to 85% solvent B (1.5% H_3PO_4, 20% HOAc, 25% MeCN in H_2O) in solvent A (1.5% H_3PO_4 in H_2O). Prep. HPLC was performed on a Waters C18 (19 ∅ × 150 mm) column at 40 °C with a flow rate of 4 mL min^{-1} and monitoring at 530 nm. The solvent used was as follows: a linear gradient elution for 15 min from 60 to 70% solvent B in solvent A. UV–vis spectra were recorded on MPS-2450 (Shimadzu) in 0.1% HCl–MeOH (from 200 to 700 nm). FAB mass spectra were measured with a JEOL SX 102 in the positive ion mode using the magic bullet (5:1 mixture of dithiothreitol and dithioerythritol) as a matrix. NMR spectra were measured at 500 MHz for ^1H spectra and at 125.78 MHz for ^{13}C spectra on JEOL LA 500 instrument in DMSO-d_6–CF_3COOD (9:1). Chemical shifts are reported relative to a TMS internal standard (δ), and coupling constants are in hertz.

2.3. HPLC analysis of anthocyanins

Dried root peels, petioles and cyanic flowers (*ca.* 5–10 mg) of 'Cherry Mate' and root peels from 'Flamboyant Sabina' were extracted with 1 mL MAW (MeOH–HOAc–H_2O, 4:1:5, v/v/v). Analysis of anthocyanins was performed on these crude extracts (20 μL each) by HPLC.

2.4. Extraction and purification of anthocyanins

Dried root peels (*ca.* 20 g) from *R. sativus* 'Cherry Mate' were immersed in 5% HOAc–H_2O (1L; HOAc–H_2O, 1:19, v/v) at room temperature for 5 h and extracted with the same solvent. The extract was adsorbed on a Diaion HP-20 (Mitsubishi Chemical's Ion Exchange Resins) column, and the column was washed with H_2O. The pigment was eluted from the column with 5% HOAc–MeOH (500 mL; MeOH–HOAc, 95:5, v/v). After concentration, the eluates were fractionated with paper chromatography (PC) using BAW. The crude fractionated pigment obtained was further purified by TLC (15% HOAc) and prep. HPLC. Each fraction was absorbed on a Diaion HP-20 column, and washed with H_2O to free from MeCN and H_3PO_4. The anthocyanins were eluted with 5% HOAc–MeOH from the column. Concentrated anthocyanin residues were dissolved in a small volume of 5% HOAc–EtOH, followed by the addition of excess Et_2O to give eight precipitated anthocyanins: pigments **1** (*ca.* 5 mg), **2** (*ca.* 3 mg), A (*ca.* 1 mg), B (*ca.* 1 mg), C (*ca.* 1 mg), D (*ca.* 10 mg), E (*ca.* 3 mg) and demalonyl pigment **1** (*ca.* 0.5 mg).

2.5. Chemical and spectroscopic analyses of purified anthocyanins

Characterization of these pigments (**1**, **2**, A–E and demalonyl pigment **1**) was carried out using the standard methods [8,9], and the data obtained are summarized in Table 1. Acid hydrolysis of pigments **1** (*ca.* 1 mg) and **2** (*ca.* 1 mg) was carried out with 2 N HCl (2 ml) at 100 °C for 1 h, to provide pelargonidin, glucose, caffeic acid and malonic acid from pigment **1**, and pelargonidin, glucose, *cis*-*p*-coumaric acid and malonic acid from pigment **2**. These compounds were confirmed by direct comparison with the authentic samples using TLC and/or HPLC. Moreover, the demalonylation of pigment **1** was carried out as the following process: pigment **1** (*ca.* 1 mg) was dissolved in 1 N HCl solution at room temperature for 5 days [10]. After disappearance of the starting material by HPLC (Fig. 1), demalonyl pigment **1** was isolated and purified from the hydrolysate by TLC (BAW and AHW). Alkaline hydrolysis of pigments **1** (*ca.* 1 mg) and **2** (*ca.* 1 mg) was carried out with 2 N NaOH solution (1 mL) under N_2 gas at ambient temperature for 15 min to give one deacylated anthocyanin, whose structure was identified to be pelargonidin 3-sophoroside-5-glucoside in comparison with the authentic sample, obtained from *Ipomoea purpurea* [11]. The structure confirmation of pigments A–E from 'Cherry Mate' was performed by the analysis of TLC, HPLC and spectrophotometric measurements with authentic anthocyanins purified from the root peels of 'Flamboyant Sabina' as the same procedure [5,12]. In order to obtain the *cis* isomer of pigment D, pigment D was dissolved in 5% HOAc–MeOH (0.1×10^{-5} M) and exposed to direct sunlight for 10 min [13] on December 8, 2006. After transformation of about 50% of the starting material was observed on TLC, the isomer was isolated and purified by TLC and HPLC to provide the pure *cis* isomer D (*ca.* 3 mg).

Table 1
Chromatographic and spectral properties of anthocyanins isolated from *Raphanus sativus* 'Cherry Mate'

Anthocyanin	R_f values (×100)				Spectral data in 0.1% HCl–MeOH				
	BAW	BuHCl	1% HCl	AHW	λ_{max} (nm)	E_{acyl}/E_{max} (%)	E_{440}/E_{max} (%)	$AlCl_3$	t_R (min)
1[a]	45	13	38	66	509, 331, 285	61	20	0	27.7
Demalonyl 1[a]	40	15	28	60	509, 327, 275	55	15	0	26.6
2[a]	47	24	56	78	512, 313, 288	69	20	0	26.4
A[b]	36	5	51	76	507, 269	–	20	0	12.6
B[b]	46	26	34	69	508, 317, 277	58	19	0	30.3
C[b]	41	15	30	67	508, 325, 275	52	17	0	30.8
D[b]	50	22	51	74	508, 315, 286	70	19	0	32.0
E[b]	48	15	46	72	509, 325, 287	72	20	0	32.6

A, Pelargonidin 3-sophoroside-5-glucoside; B, pelargonidin 3-[2-(glucosyl)-6-(*trans-p*-coumaroyl)-glucoside]-5-glucoside; C, pelargonidin 3-[2-(glucosyl)-6-(*trans*-feruloyl)-glucoside]-5-glucoside; D, pelargonidin 3-[2-(glucosyl)-6-(*trans-p*-coumaroyl)-glucoside]-5-(6-malonyl-glucoside); E, pelargonidin 3-[2-(glucosyl)-6-(*trans*-feruloyl)-glucoside]-5-(6-malonyl-glucoside).

[a] 1: Pelargonidin 3-[2-(glucosyl)-6-(*trans*-caffeoyl)-glucoside]-5-(6-malonyl-glucoside); 2: pelargonidin 3-[2-(glucosyl)-6-(*cis-p*-coumaroyl)-glucoside]-5-(6-malonyl-glucoside), demalonyl 1: pelargonidin 3-[2-(glucosyl)-6-(*trans*-caffeoyl)-glucoside]-5-glucoside.

[b] Identification with anthocyanins from root peels of 'Flamboyant Sabiba' [5,11].

3. Results and discussion

3.1. Identification of known anthocyanins

For identification of known anthocyanins A–E (Fig. 1) isolated from the 'Cherry Mate', we used purified anthocyanins from the roots of 'Flamboyant Sabina' as a comparative standard [5,12]. As a result, we were able to confirm the structures of anthocyanins A–E as pelargonidin 3-sophoroside-5-glucoside, pelargonidin 3-[2-(glucosyl)-6-(*trans-p*-coumaroyl)-glucoside]-5-glucoside, pelargonidin 3-[2-(glucosyl)-6-(feruloyl)-glucoside]-5-(glucoside), pelargonidin 3-[2-(glucosyl)-6-(*trans-p*-coumaroyl)-glucoside]-5-(6-malonylglucoside) and pelargonidin 3-[2-(glucosyl)-6-(feruloyl)-glucoside]-5-(6-malonylglucoside), respectively (Table 1). Analysis of the extracts from the 'Cherry Mate' root peels, petioles and cyanic flowers with HPLC led to the determination of five known pigments A–E, in addition to another major anthocyanin (pigment **1**), whose structure was presumed to be caffeoyl pelargonidin 3-sophoroside-5-glucoside by Giusti et al. [5] (pigment number 2 in their paper) (Fig. 1). However, we found that the structure of pigment **1** is different from the structure proposed by Giusti, and malonyl group was supposed to be present in this pigment. Moreover, using HPLC to analyze the extracts from the 'Cherry Mate' petiole and cyanic flowers, we detected another major anthocyanin (pigment **2**) which was scarcely present in the root peels. Details of the structural elucidations of new pigments are carried out as follows.

3.2. Pigment *1* and its demalonyl derivative

The FAB mass spectrum of pigment **1** showed a molecular ion peak $[M]^+$ at 1005 *m/z*, corresponding to the molecular formula $C_{45}H_{49}O_{26}$ (1005.251). Full assignment of 2D-COSY, NOEDIF, $^1H-^{13}C$ HMQC and $^1H-^{13}C$ HMBC spectra are shown in Table 2. The ^1H NMR spectrum of pigment **1** demonstrated the presence of three molecules of glucose and one molecule each of pelargonidin, caffeic acid and malonic

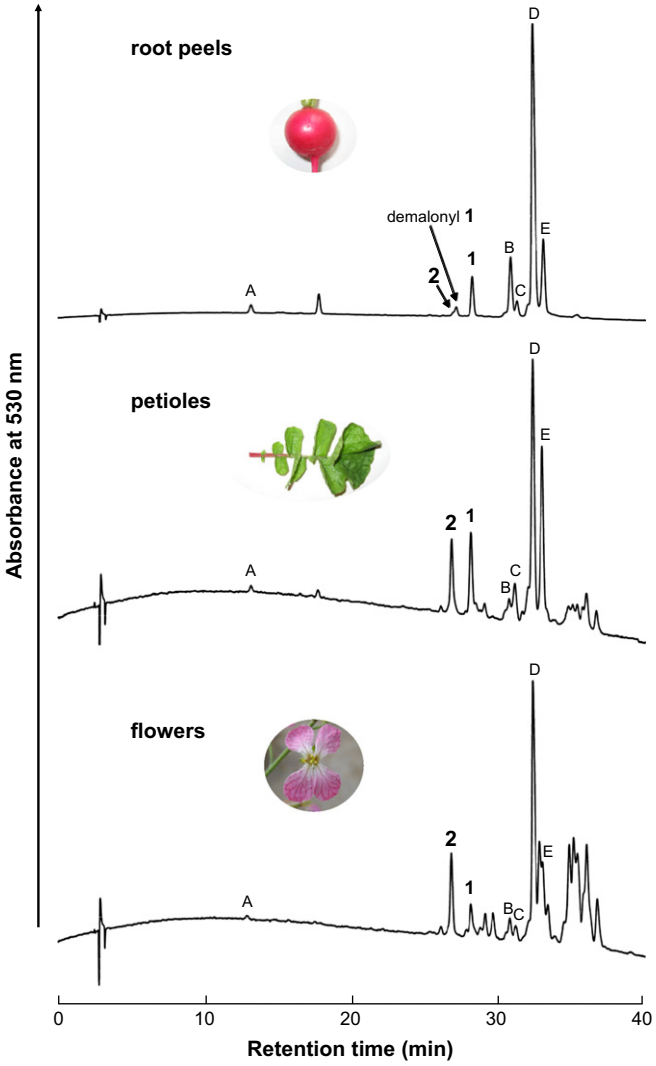

Fig. 1. HPLC profiles for the anthocyanins in the root peels, petioles and flowers of *Raphanus sativus* 'Cherry Mate' pigments A–E are same as in Table 1.

Table 2
^{1}H and ^{13}C NMR spectroscopic data (δ) of acylated anthocyanins from *Raphanus sativus* L. (500 MHz in DMSO-d_6–CF_3CO_2D, TMS as an internal standard) [coupling constants (J in Hz) in parentheses]

	1		2	D
	^{1}H	^{13}C	^{1}H	^{1}H
Pelargonidin				
2		163.0		
3		144.5		
4	8.85 s	132.8	8.59 s	8.81 s
5		155.0		
6	7.02 d(1.9)	104.9	6.87 brd(1.8)	7.00 brs
7		167.7		
8	7.13 d(1.9)	96.5	6.93 brd(1.8)	7.07 brs
9		155.4		
10		111.9		
1′		119.4		
2′	8.62 d(9.2)	135.4	8.51 d(9.2)	8.60 d(9.2)
3′	7.12 d(9.2)	117.1	7.11 d(9.2)	7.11 d(9.2)
4′		165.4		
5′	7.12 d(9.2)	117.1	7.11 d(9.2)	7.11 d(9.2)
6′	8.62 d(9.2)	135.4	8.51 d(9.2)	8.60 d(9.2)
Glucose A (3-glucoside)				
1	5.67 d(7.3)	99.5	5.64 d(7.3)	5.67 d(7.7)
2	4.10 t*(7.9)	79.6	4.08 t*(8.3)	4.09 t*(8.0)
3	3.74 t*(8.9)	76.3	3.68 t*(8.7)	3.74 t*(8.7)
4	3.53 t*(8.6)	69.7	3.27 m	3.47 m
5	3.98 m	73.9	3.82 m	4.00 t*(9.2)
6a	4.31 dd(6.4, 12.2)	63.0	4.31 m	4.29 dd(6.6, 12.5)
6b	4.44 brd(11.6)		4.44 d(10.4)	4.42 brd(12.5)
Glucose B (2″-glucosyl)				
1	4.76 d(7.7)	103.2	4.69 d(8.0)	4.75 d(7.7)
2	3.01 t*(8.3)	74.4	2.93 t*(8.6)	2.99 t*(8.5)
3	3.15 t*(9.3)	76.5	3.07 t*(8.9)	3.05 t*(9.3)
4	3.06 t*(9.2)	70.0	2.98 t*(8.9)	2.85 m
5	2.86 ddd(2.2, 6.1, 9.6)	77.2	2.71 m	3.14 t*(8.9)
6a	3.28 m	61.0	3.21 dd(6.1, 12.2)	3.26 m
6b	3.38 m		3.48 brd(10.5)	3.52 m
Glucose C (5-glucoside)				
1	5.20 d(7.6)	101.6	5.20 d(7.6)	5.19 d(7.6)
2	3.56 t*(8.4)	73.2	3.54 t*(8.5)	3.56 t*(8.4)
3	3.43 t*(9.0)	75.9	3.39 t*(8.9)	3.40 t*(7.0)
4	3.28 t*(8.9)	69.6	3.27 t*(9.8)	3.28 m
5	3.80 ddd(2.1, 7.0, 9.8)	74.3	3.36 m	3.80 t*(9.2)
6a	4.12 m	64.1	3.95 m	4.06 m
6b	4.44 brd(11.6)		4.36 m	4.39 brd(12.5)
Hydroxycinnamic acid				
1		125.6		
2	6.91 brd(2.1)	115.6	7.28 d(8.9)	7.33 d(8.9)
3		145.7	6.46 d(8.9)	6.71 d(8.9)
4		148.4		
5	6.73 d(8.2)	115.8	6.46 d(8.9)	6.71 d(8.9)
6	6.87 dd(2.1, 8.2)	121.1	7.28 d(8.9)	7.33 d(8.9)
7a	7.34 d(15.9)	145.5	6.46 d(12.8)	7.37 d(15.9)
8b	6.19 d(15.9)	113.7	5.68 d(12.8)	6.25 d(15.9)
9		166.7		
Malonic acid				
1		167.0		
2	3.39 s	41.3	3.41 s	3.39 s
3		168.1		

s = Singlet, d = doublet, brd = broad doublet, t* = distorted triplet, m = multiplet, dd = double doublet, ddd = double double doublet.

acid. The aromatic protons of pelargonidin and caffeic acid in this pigment were assigned by analysis of the 2D-COSY spectrum (Table 2). The proton signals of the sugar moiety were observed in the region of δ 2.86–5.67 (Table 2). The signals of three anomeric protons appeared at δ 5.67 (1H, d, $J = 7.3$ Hz, Glu A), δ 4.76 (1H, d, $J = 7.7$ Hz, Glu B) and δ 5.20 (1H, d, $J = 7.6$ Hz, Glu C), and the assigned neighboring diaxial hydrogens had large coupling constants (*ca.* 7–9 Hz). Therefore, these three glucoses must be β-D-glucopyranose. The characteristic four protons shifted to the lower magnetic fields were assigned to two methylenes ($-CH_2-$) of Glu A (δ 3.98 and 4.31) and Glu C (δ 4.12 and 4.44). These results revealed that the OH-6 of Glu A and C was acylated by two acid residues (caffeic or malonic acid). Moreover, a signal appeared at δ 4.10 (t, $J = 7.9$ Hz, H-2 of Glu A) was easily correlated to the proton H-1 of Glu A. Thus, this proton was assigned to the H-2 of Glu A. The signal of the anomeric proton of Glu B correlated to the signal of the H-2 proton of Glu A in the NOEDIF spectrum. These results suggested that Glu B attached to OH-2 of Glu A through a glucosidic bond, and formed a sophorose unit. NOEDIF and HMBC spectra were used to confirm the sites of attachment of hydroxycinnamic acid, the sugar and pelargonidin aglycon, respectively (Fig. 2). The signals of the anomeric protons of Glu A and Glu C were correlated to the signals of the C-3 and C-5 carbon of pelargonidin in the HMBC spectra, and also to the signals of proton H-4 and H-6 of pelargonidin in the NOEDIF spectra. Moreover, the weak NOEDIF effect was observed between the anomeric proton of Glu A and the proton of α of caffeic acid moiety, indicating that the caffeic acid was bonded to the OH-6 of Glu A. The malonic acid was determined to be bonded to the OH-6 of Glu C, based on the downfield shift of the methylene protons of Glu C. Therefore, pigment **1** is pelargonidin 3-*O*-[2-*O*-(β-D-glucopyranosyl)-6-*O*-(*trans*-caffeoyl)-β-D-glucopyranoside]-5-*O*-(6-*O*-malonyl-β-D-glucopyranoside) (Fig. 2), a novel anthocyanin [14–16].

Although Giusti et al. [5] assumed that pigment **1** would be caffeoyl pelargonidin 3-sophoroside-5-glucoside, we were able to revise the structure of pigment **1** to be malonylated with 6-OH of the glucose of pelargonidin at 5-position. For further confirmation of the structure of pigment **1**, we obtained

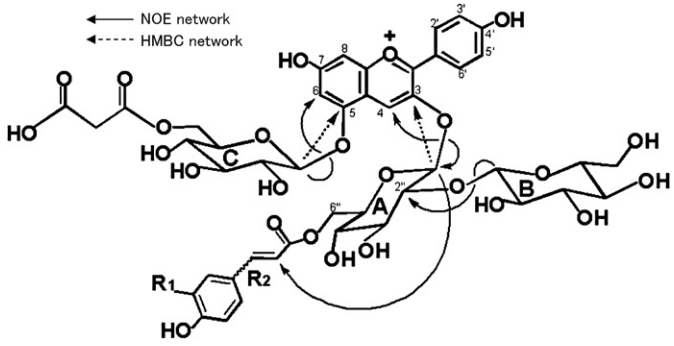

Fig. 2. New anthocyanins from *Raphanus sativus* 'Cherry Mate'. Arrows highlight important NOE and HMBC networks. Pigment **1**; R1 = OH, R2 = *trans*, pigment **2**; R1 = H, R2 = *cis*.

the demalonyl derivative of **1** by hydrolysis of pigment **1**, and found that the hydrolysis product, pelargonidin 3-caffeoylsophoroside-5-glucosde, was identical with the structure proposed by Giusti. The properties of demalonyl derivative of **1** are shown in Table 1 and Fig. 1.

3.3. Pigments **2** and D

The FAB mass spectra of pigments **2** and D gave their $[M]^+$ at 989 and 989 *m/z*, corresponding to the mass calculated for $C_{45}H_{49}O_{25}$ (929.246) and $C_{45}H_{49}O_{25}$ (929.246), respectively. ^{1}H NMR spectrum of pigment D showed the presence of three molecules of glucose and one molecule each of pelargonidin, *trans-p*-coumaric acid and malonic acid (Table 2). Pigment D was directly compared and identified with the data of pelargonidin 3-[2-(glucopyranosyl)-6-(*trans-p*-coumaroyl)-glucopyranoside]-5-(malonyl-glucopyranoside) isolated from the root peels of 'Flamboyant Sabina' [5,12] (Table 1). The ^{1}H NMR spectrum of pigment **2** was identical with that of pigment D except for the signals of *p*-coumaroyl moiety (Table 2). Particularly, the chemical shifts of the olefinic protons were shifted to a higher magnetic field at δ 5.68 and 6.46 with smaller coupling constants ($J = 12.8$ and 12.8 Hz) in comparison with those (δ 6.25, 15.9 Hz and δ 7.37, 15.9 Hz) of pigment D. Since the configuration of *p*-coumaric acid was confirmed to be *cis*, pigment **2** was determined to be pelargonidin 3-*O*-[2-*O*-(β-D-glucopyranosyl)-6-*O*-(*cis-p*-coumaroyl)-β-D-glucopyranoside]-5-*O*-(6-*O*-malonyl-β-D-glucopyranoside) (Fig. 2), which is a novel anthocyanin in plants [14–16].

4. Conclusion

From the chemotaxonomical point of view, two typical anthocyanidin glycoside types have been advanced in this family. One of the types is the acylated 3-sophoroside-5-glucosides of cyanidin and pelargonidin from the root of *R. sativus* [1–7], leaves and/or stems of *Brassica oleracea* [3,17–22], *Brassica campestris* [23,24] and *Sinapis alba* [25]. The other is acylated 3-sambubioside-5-glucoside of cyanidin and pelargonidin from the flowers of *Matthiola incana* [26,27], *Orychophragonus violaceus* [9], *Cheiranthus cheiri*, *Lunaria annua* and *Lobularia maritima* [28,29], and the leaves and stems of *Arabidopsis thaliana* [30]. Research on the former type of anthocyanins has, for the most part, made use of colorants isolated from roots, leaves and stems which have high concentrations of pigments. However, anthocyanins from flowers have not been investigated in genus *Raphanus*, because of the inedibility. This experiment has proven that acylated anthocyanidin 3-sophoroside-5-glucoside also exists in the flowers of Cruciferae. In the flowers and petioles, some anthocyanins not found in the root peels were detected (Fig. 1). Pigment **2** was found with an especially high concentration ratio in flowers. Pigment **2** is formed when *p*-coumaroyl moiety of pigment D is isomerized from *cis* to *trans* form. We suppose this process is caused by sunlight (especially UV), therefore, pigment **2** is found in greater quantities in the plant organs, such as flowers and petioles, with greater exposure to the sun.

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